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INVESTIGATION ON THE NEUROPROTECTIVE EFFECTS OF HESPERIDIN ON BEHAVIOURAL ACTIVITIES IN 6-OHDA INDUCED PARKINSON MODEL

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ABSTRACT

The present study was carried out to evaluate the neuroprotective activity of hesperidin on Parkinson induced experimental animals. Parkinson disease (PD) is one of the neurodegenerative disease and oxidative stress plays a vital role in its causation. Group 1: Animals were treated normally and served as controls. Group 2: Rats were infused with 6-hydroxydopamine (8µg/2µl in 0.1% ascorbic acid-saline) in right striatum once and were maintained for the development of Parkinson's disease for 45 days. Group 3: Rats were infused with 6-hydroxydopamine (8µg/2µl in 0.1% ascorbic acid-saline) in right striatum once and were maintained for development of Parkinson's disease for 3weeks. From 22nd day Hesperidin (50 mg/kg b.w) dissolved in distilled water was given for next 24 days. Group 4: Rats were infused with 6-hydroxydopamine (8µg/2µl in 0.1% ascorbic acid-saline) in right striatum once and were maintained for the development of Parkinson's disease for 3weeks. On the 22nd day Hesperidin (50mg/kg b.w) and L-Dopa (100mg/kg b.w) dissolved in distilled water was given for the next 24 days. Group5:Rats were infused with 6-hydroxydopamine (8µg/2µl in 0.1% ascorbic acid-saline) in right striatum for once and the rats were maintained for the development of Parkinson's disease for 3 weeks. On the 22nd day (L-DOPA 100mg/kg b.w) were given for next 24days. Neurological activities such as Grip test, rotational test, Swing and climb test, catalepsy etc., were studied. After 45 days body weight was noted and the animals were killed and blood glucose, protein and triglycerides in serum of rat animals were estimated. The deficits in behavioral activity due to 6-OHDA lesioning were significantly restored by hesperidin and L-Dopa combination. This study indicates that a combination of hesperidin and L-Dopa might be helpful in attenuating 6-OHDA induced lesioning in rats.

KEYWORDS: Hesperidin, L-Dopa, 6-hydroxydopamine(6-OHDA), oxidative stress, behavioural parameters, neuroprotection, Parkinson disease (PD).

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INTRODUCTION

Parkinson disease is a slow progressive neurodegeneration disease caused when a small group of brain cells die which control the body movements. This disease was first described by James Parkinson in 1817. It's symptoms are clinically characterized by bradykinesia, resting, tremor, rigidity and postural instability. Pathological features of PD include loss of dopamine neurons in substantia nigra and the presence of Lewy bodies in surviving dopamine neurons.[1] The available treatments are Levodopa, Carbidopa, Amantadine, Orphenadrine, Selegeline, Pergola, Benztropine and many more. These drugs effectively reverse the symptoms of Parkinson and improve the level of dopamine. The available drug treatments for PD possess various side effects like nausea, vomiting, respiratory disturbances, hallucinations, anxiety arrhythmia and insomnia. The major side effects of long term therapy with levodopa are wearing off phenomenon, on-off phenomenon and dyskinesia. 6-hydroxydopamine (6-OHDA) is a specific neurotoxin for catecholaminergic pathways.[3-4] Being structurally similar to the catecholamines, it uses the respective transport system to enter the neurons and destroys them. 6-OHDA has been reported to produce some of the behavioral, biochemical and pathological changes that are encountered in PD[5] and because of established stereotaxic techniques and relatively low maintenance costs, is currently the most commonly used animal model for the disease.[6] These toxic effects of 6-OHDA are attributed to the formation of various oxidants and free radicals[7,] lipid peroxidation[8], protein damage and amino acid modifications.[9] In addition, studies have demonstrated that 6-OHDA leads to reduction in glutathione (GSH) content, superoxide dismutase (SOD), catalase (CAT) activities, and an increase in lipid peroxidation in striatum.[10-15] PD is an age related disorder more common in the aged population than in the young. The disease is accompanied by the symptoms of rest tremor, bradykinesia, rigidity, stoped posture and instability. Exact cause of this disease still remains a mystery, despite the known role of oxidative stress, free radical formation[16] genetic susceptibility[17], programmed cell death[18] and another unknown factor, which might be endogenous (or) exogenous.[19] The disease progresses slowly and can ultimately produce complete akinesia. The neuropathology of the disease is based on the depigmentation and cell loss in the dopaminergic nigrostriatal tract of the brain[20] with a corresponding decrease in striatal dopamine content. PD is a multicentric neurodegenerative disorder that affects several neuronal structures in addition to the substantia nigra, including the enteric nervous system.[21-22] Recently many studies have focused their effect on the protective effects of plant derived compounds on diverse neuropathological conditions. Hesperidin, a bioflavonoid is an abundant and inexpensive by-product of citrus family. Among the many benefits attributed to flavonoids some are proposed to have reduced risk of cancer, heart diseases, asthma and stroke. They play a special role in protecting brain. *In-vitro* antioxidant property of hesperidin has been proven that hesperidin is capable of scavenging free radicals more efficiently as the concentration increases and it may be suggested that hesperidin has great importance as therapeutic agent in preventing (or) slowing the progress of aging and age associated oxidative stress related degeneration disease.[23]Structure activity relationship of hesperidin to their antioxidant property showed that the effects of anti-oxidative activity of hesperidin depend on the number and order of OH groups and the presence of C4'-C8' double bond conjugated with a 4-keto group in the flavonoid structure.[24] Anti-hypercholestrolamic activities of hesperidin in CCl_4 induced hypercholsterolamic rats have been reported.[25] Hesperidin is an effective scavenger of free radicals where this property helps to protect the lungs from nicotine-induced toxicity.[26] As a powerful antioxidant , hesperidin has demonstrated remarkable protection against carcinogens and acts as a scavenger that searches for and engulfs the free radicals that cause the cell damage produced by unstable oxygen molecules.[27] Hesperidin and lipoic acid exhibited protective effects against sodium arsenite induced acute toxicity in liver and kidney of mice. These

compounds may potentially play an important role in the protection of population chronically exposed to arsenic.[28] Hesperidin protects against ischemic reperfusion induced behavioral alterations, biochemical alterations and mitochondrial dysfunctions in the brain and the protective effects of hesperidin may have involvement of nitric oxide pathway.[29]Hesperidin treatment could reduce cerebral damage due to induced stroke in the rat brain due to the reduction of free radicals and associated neuro-inflammation.[30] Hesperidin alternates neuronal damage induced by rotenone by reducing oxidative stress, mitochondrial dysfunction and ameliorating apoptosis.[31] Toxicity of Doxorubicin on rat heart was mediated through oxidative stress mechanisms and pretreatment with hesperidin reversed most of the negative effects induced by DOX.[32]Taking into account of all the beneficial effects of hesperidin, the aim of the study was to evaluate its neurobehavioral activity, and to study the levels of glucose, protein and triglycerides in serum samples of Parkinson animal model .

MATERIALS AND METHODS

6-OHDA, ascorbic acid, Hesperidin and Apomorphine were purchased from sigma Aldrich. All other chemicals used were of analytical grade.

Experimental Animals

Adult male wistar rats (145-150g) were used for the study. Animals were purchased from Tamilnadu Veterinary and Animal Science University, Madhavaram, Chennai and were housed under controlled temperature provided with food and water ad libitum. The protocol was approved by the institutional animal ethics committee of the Saveetha University, Chennai.
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(i) Experimental protocol

The animals were divided into 5 groups, each containing 6 animals.

Group 1: Animals were treated normally and served as controls.

Group 2: Rats were infused with 6-hydroxydopamine (8µg/2µl in 0.1% ascorbic acid-saline) in right stratum once and were maintained for the development of Parkinson's disease for 45 days.

Group 3: Rats were infused with 6-hydroxydopamine (8µg/2µl in 0.1% ascorbic acid-saline) in right stratum once and maintained for the development of Parkinson's disease for 3weeks. On the 22nd day, Hesperidin (50 mg/kg b.w) dissolved in distilled water were given for next 24 days.

Group 4: Rats were infused with 6-hydroxydopamine (8µg/2µl in 0.1% ascorbic acid-saline) in right stratum once and were maintained for development of Parkinson's disease for 3weeks. On the 22nd day, Hesperidin (50mg/kg b.w) and L-Dopa (100mg/kg b.w) dissolved in distilled water were given for next 24 days.

Group 5: Rats were infused with 6-hydroxydopamine (8µg/2µl in 0.1% ascorbic acid-saline) in right stratum for once and were maintained for the development of Parkinson's disease for 3 weeks. On the 22nd day (L-DOPA 100mg/kg b.w) was given for next 24days.

6-OHDA induced lesions

All animals in the experimental groups were anaesthetized with Ketamine (100mg/kg b.w) (i.p) and Xylazine (10 mg/kg b.w) (SC). Each animal was mounted on a stereotaxic apparatus, the skin overlying the skull was cut to expose it, and the co-ordinates for the stratum [33] were measured accurately (anterior-posterior 0.5 mm, lateral 2.5 mm, dorso-ventral from dura) with the tooth bar set at 0 mm. Thereafter, all animals in the experimental groups were lessened by injecting (8µg/2µl in 0.1% ascorbic acid-saline) in the right striatum, while the Group I served as a control. The injections were made manually, with the help of a Hamilton syringe, through the burr holes made on the skull surface in the experimental groups. The injection rate was 1.0µl/min and the needle was kept in place for an additional 1 min before being slowly retracted.

Post operative care

Recovery from anesthesia took approximately 4-5h. The rats were kept in a well-ventilated room at 25±3ºC in individual cages until they gained full consciousness; they were then housed together in groups of 6 animals per cage. Food and water mixed with 1ml of Ibuprofen was kept inside the cages for two days, allowing animals easy access, without physical trauma due to overhead injury. Animals were then treated normally, food, water and the bedding of the cages were changed twice per week, as usual.

BODY WEIGHT

The body weight of each animal was weighed before and after the treatment.

(ii) BEHAVIOURAL STUDIES

All the behavioral studies were performed at room temperature in a calm room without any outside interference.

a.) ***Grip Test***

Grip strength was performed according to the method.[34] The animals were trained for the grip test before the experiment. After 21 days of lesioning, the animals were again tested. The apparatus has a string of 50cm-length, pulled out between two vertical supports and evaluated according to the following scale: 0-fall off, 1-hangs onto the string by two forepaws, 2 as for one attempts to climb on string, 3- hangs onto the string by two forepaws plus one (or) both hind limbs, 4-hangs onto string by forepaws plus tail wrapped around the string, 5-escapes. The highest reading of successive trials was taken from each animal.

b.) Rotational test

The rotational test was performed by the administration of apomorphine (0.5mg/kg b.w) after three weeks of lesioning to rats. The contra-lateral body rotation induced by the apomorphine was observed.

c.) Swing test

The body swing test was performed after 21 days of induction of Parkinson disease. We adapted the test for unilaterally lesioned rats.[35] Each rat was held 1 cm from the base of its tail and suspended vertically 1cm above the ground. Swings were recorded whenever the rat moved its head right and left of the vertical axis.Two examiner performed the test, one hold the rat while the other determined direction and frequency of the movement and timed the session. The number of swings to each side was expressed as a score. Vertical position: score 1, left swing: score 2 , Right swing: score 3, holding the tail: score 4, climbing up: score 5.

d.) Catalepsy

The front paws of the rats were placed alternately on a 5 cm high block, if the rat failed to correct the posture within 15 seconds a score of 0.5 was assigned. Thus, the score reflects total catalepsy.[36]

(iii) BIOCHEMICAL PARAMETERS

a.)Estimation of blood glucose

Blood glucose level was estimated by the method of glucose oxidase.[37]

b.)Estimation of Protein

The protein content was Estimated according to the method of Lowry *et* al.[38]

c.) Estimation of Triglycerides

Triglycerides content was estimated by the method of Rice.[39]

STATISTICAL ANALYSIS

All values are expressed as Mean ±SD. Statistical comparisons were performed by one way ANOVA and the data were evaluated using Graph Pad Prism. $^{*}P< 0.05$ was considered as statistically significant.

RESULTS

(i)Table 1 shows the effect of hesperidin on body weight of experimental animals. It is observed that the body weight decreased due to systemic administration of 6-OHDA when compared to control. Significant reversal was noted in 6-OHDA induced groups treated with hesperidin, hesperidin +L-Dopa and L-Dopa.

Table 1
Effect of Hesperidin on Body weight in experimental rats

Particulars	Body weight of animals/gm				
	Group I	Group II	Group III	Group IV	Group V
Initial weight	153.66±0.51	153.0±1.09^{aNS}	152.16±0.40^{bNS}	152.83±0.75^{bNS}	152.50±1.51^{bNS}
Final weight	158.16±1.16	146.0±1.89^{a*}	154.50±1.04^{b*}	158.0±0.89^{b*}	156.50±1.37^{b*}

**P<0.001, **P<0.01, ***P<0.05, NS-Non significant.*
a- comparison between group II and group I.
b- comparison between group III, IV, V and group II

(ii)Table 2 reveals the movement co-ordination impairment of experimental rats. Systemic administration of 6-OHDA caused impaired ability to initiate movement which has been proved by grip test, rotation test, swing test and catalepsy test. 6-OHDA induced groups significantly reduced the behavioural activities (*P<0.001) at a dose of (8µg/2µl in 0.1% ascorbic acid-saline). Significant reversal was observed, with the administration of hesperidin (50mg/kg b.w)+L-Dopa (100mg/kg b.w). The maximal decrease (*P<0.001) in behavioural changes was observed in the groups receiving hesperidin (50mg/kg b.w)+L-Dopa (100mg/kg b.w) combination.

Table 2
Effect of Hesperidin on Behavioural tests in experimental rats

Particulars	Behavioural tests				
	Group I	Group II	Group III	Group IV	Group V
Grip test (Score)	4.50±0.54	1.66±0.51^{a*}	2.66±0.51^{b**}	4.00±0.63^{b*}	2.33±0.51^{b***}
Rotation test/min	6.5±0.54	27.16±0.75^{a*}	26.0±0.63^{b**}	15.83±1.16^{b*}	26.16±0.75^{b***}
Swing test (Score)	4.66±0.51	1.50±0.54^{a*}	2.50±0.83^{b**}	3.83±0.40^{b*}	2.16±0.40^{b***}
Catalepsy (Score)	0	4.16±0.25^{a*}	3.16±0.68^{b**}	0.83±0.25^{b*}	3.50±1.04^{b***}

**P<0.001, **P<0.01, ***P<0.05, NS-Non significant.*
a- comparison between group II and group I.
b- comparison between group III, IV, V and group II

(iii) Table 3 shows changes in biochemical parameters after administration of 6-OHDA 6-OHDA induced groups showed significantly (*P<0.001) low levels of glucose and triglycerides at a dose of (8µg/2µl in 0.1% ascorbic acid-saline). Significant reversal in 6-OHDA induced groups was observed, with the administration of hesperidin (50mg/kg b.w), +L-Dopa (100mg/kg b.w). The maximal increase (*P<0.001) in biochemical parameters was observed in the groups receiving hesperidin (50mg/kg b.w) + L-Dopa (100mg/kg b.w).

Table 3
Effect of Hesperidin on Glucose, Triglycerides and Protein in experimental rats

Particulars	Group I	Group II	Group III	Group IV	Group V
Glucose (mg/dl)	110.81±2.57	95.10±0.80^{a*}	97.16±0.75^{b**}	108.16±1.12^{b*}	97.05±0.81^{b***}
Triglycerides (mg/dl)	74.73±0.67	63.43±0.44^{a*}	65.66±1.50^{b**}	71.83±0.93^{b*}	64.83±1.83^{b***}
Protein (g/dl)	6.33±0.13	6.06±0.10^{a*}	6.13±0.13^{bNS}	6.26±0.02^{b**}	6.16±0.13^{bNS}

**P<0.001, **P<0.01, ***P<0.05 . NS-Non significant.*
a- comparison between group II and group I.
b- comparison between group III, IV, V and group II

DISCUSSION

Parkinson's disease is a neurodegenerative disorder characterized by the selective loss of dopamine (DA) neurons in the substantia nigra pars compacta. Current treatment for parkinson's disease (PD) is based on dopamine replacement therapy, but this leads to long term complications, including dyskinesia. Flavonoids play an important role and a safer alternative to the treatment of neurodegenerative disorders including parkinsonism. In the present study, we have evaluated the effect of hesperidin, a flavonoid on 6- OHDA induced Parkinson. 6-OHDA is a widely accepted animal model for PD. 6-OHDA induced behavioral studies in rats are being used for evaluating the drugs for their anti-parkinsonism effects. In this study hesperidin was screened for its effect on 6-OHDA induced Parkinson. Hesperidin at a dose of 50mg/kg b.w exhibited a pharmacological effect which is slightly significant than standard drug (L-Dopa) and further potentiation was observed with L-Dopa and hesperidin combination therapy.Epidemiological studies have shown beneficial effects of flavonoids on neurodegeneration in particular. Flavonoids can protect the brain by their ability to modulate intracellular signals promoting cellular survival. Neuroleptics like 6-OHDA exert multiple events on dopaminergic signaling and produce DA related behavioral changes. It can be hypothesized from the study that hesperidin ameliorates the symptoms of 6-OHDA behavioural activities in rats. The mechanism by which the amelioration takes place may be attributed to one (or) more pharmacological/biochemical mechanism, hesperidin may enhance the bioavailability of circulatory dopamine by upregulation of dopamergic signaling and hesperidin may enhance the bioavailabity of L-Dopa by inhibiting Dopa-decarboxylase activity like that of carbidopa .[40-41] Different age groups suggest that there might be an association between oxidative stress and the age progression. The possibility of counteracting oxidative stress by a pool of proper antioxidants plus an appropriate diet, may have a real health benefit to reduce morbidity and perhaps increase the healthy, useful life span of an individual. [42]This paper emphasizes to explore the effect of hesperidin on 6-OHDA induced behavioral studies in rats. In the present study the effect of hesperidin on extrapyramidal symptoms such as behavioral parameters, which are key parameters found in PD was studied. Body weight has been decreased in group II, when compared to control. Body weight is near normal in the treated groups when compared to control. Hesperidin at a dose of (50mg/kg b.w) combined with L-dopa (100mg/kg b.w) exhibited significant changes in the behavioural activities as well as blood glucose, TG and protein. Hesperidin showed comparable neuroprotective actions with that of standard drug L-dopa. Hesperidin at a dose of 50mg/kg b.w significantly reversed the 6-OHDA inhibited behavioral activity. The standard drug L-Dopa and Hesperidin which is highly significant reversed the 6-OHDA inhibited behavioral activity. Combination of Hesperidin (50mg/kg b.w) and L-Dopa (100mg/kg b.w) significantly, increase the exploratory behavior activities like grip test, rotational test, swing test and catalepsy in 6-OHDA induced rats. Blood glucose, Triglycerides and protein were shown to be normal in all the groups except the induced group (Group II). From the observations of this study, it could be envisaged, that the protective effect of hesperidin against symptoms of PD may be due to regulation in neurotransmitters such as dopamine, serotonin, nor-epinephrine and epinephrine which may play an important role in protection of neurobehavioral activities and antioxidant properties. Further biochemical studies have to be conducted for their effect on Parkinson disease.

CONCLUSION

Hesperidin was found to possess therapeutic effect against Parkinson disease in 6-OHDA induced animal model. These findings may have important implications in the use of hesperidin for the prevention of PD. However, further research involving the genes responsible for Parkinson disease is needed to be validated for hesperidin to act as a new therapeutic agent.

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